Janzen EG and Blackburn BJ (1968) Detection and identification of short-lived free radicals by an electron spin resonance trapping technique. *Journal of the American Chemical Society* 90: 5909–5910.

Kroll C, Osterloh K, Mäder K and Borchert H-H (1998) On-line coupled HPLC-ESR: Application for separation and characterization of organic radicals. *Archiv der Pharmazie – Pharmaceutical and Medicinal Chemistry* 331 (2): 40.

Osterloh K, Kroll C, Mäder K, Borchert H-H, Sammler B and Kirmse C (1998) HPLC und Elektronenspin-resonanz-Spektroskopie on-line gekoppelt [On-line Coupling of HPLC and Electron Spin Resonance Spectroscopy] (German). *GIT Labor-Fachzeitschrift* 42: 24–25.

Poole CP (1983) *Electron Spin Resonance*, 2nd edn. New York: John Wiley.

Poole CP and Farach HA (1994) *Handbook of Electron Spin Resonance: Data Sources, Computer Technology, Relaxation, and Endor*. Berlin: Springer.

Sugata R, Iwahashi H, Ishii T and Kido R (1989) Separation of polyunsaturated fatty acid radicals by high-performance liquid chromatography with electron spin resonance and ultraviolet detection. *Journal of Chromatography* 487: 9–16.

Suzuki S, Nakazawa H, Fujita M, Ono S, Suzuki M, Takitani S, Sonoda M and Sakagishi Y (1992) Flow analysis of UV-irradiated chemicals by chemiluminescence and electron spin resonance spectroscopy. *Analytica Chimica Acta* 261: 39–43.

Detectors: Evaporative Light Scattering

R. P. W. Scott, Avon, CT, USA

Copyright © 2000 Academic Press

The evaporative light-scattering detector evolved from the early work of Charlseworth and MacRae. The device consists of a spray system that continuously atomizes the column eluent into small droplets. The droplets evaporate, leaving the solute as fine particulate matter suspended in the atomizing gas. In practice, the column eluent passes into a concentric nebulizer where it is nebulized in a hot stream of gas that may be air or, if so desired, an inert gas such as helium or argon. The suspended particulate matter is then made to pass through an intense light beam from a source such as a helium–neon laser. The light scattered by the particles is viewed at 45° to the incident beam by means, for example, of a pair of properly placed optical fibres. The scattered light that enters the fibres is transmitted to a photomultiplier, the output of which is electronically processed and passed either to a computer acquisition system or to a potentiometric recorder. The evaporative light-scattering detector might be considered to be a form of transport detector where the transport medium is the nebulizing gas. A diagram of the light-scattering detector is shown in **Figure 1**.

The column eluent enters the centre orifice of a dual, concentric jet nebulizer where it meets a heated stream of nebulizer gas from the surrounding annular orifice. The gas flow rate is adjusted to provide a jet velocity that is just above the speed of sound. The stream of droplets that are produced normally have a relatively wide range of size distribution and pass down a heated tube, called the drift tube. In this tube the solvent evaporates, leaving the solute as residual solid particles still carried in the gas stream. It is clear that this type of detector cannot function effectively if solid involatile buffers are used in the mobile phase. After passing through the laser beam, the gas containing the solvent vapour and particles is aspirated through a simple water pump which safely disposes of both the solvent vapour and the solutes. The laser is employed as a convenient source of high intensity light and its coherence does not appear to confer any particular advantage on the detection system.

The amount of scattered light that is collected is related to the diameter of the particles, the wavelength of the incident light and the angle at which it is collected. It is not linearly related to the concentration of solute in the mobile phase, but varies as either the power or the exponent of the solute concentration. For a given set of operating conditions the droplet size will remain sensibly constant during the development of a chromatogram. Now the average diameter of the solid particles produced will be the average size of the droplets multiplied by the cube root of the solute concentration. Thus, if the solute has a concentration of 10^{-6} g mL^{-1}, the solute particles will be 100 times smaller than the size of the droplets and, assuming a common value for the mean diameter of the droplets of 20 μm, the solute particles will be 0.2 μm in diameter. Thus the mean particle diameter is of approximately the same order of magnitude as the wavelength of the scattered light.

Physical Properties of the Nebulizer

The intensity of the scattered light will be determined, among other factors, by the diameter of the solid

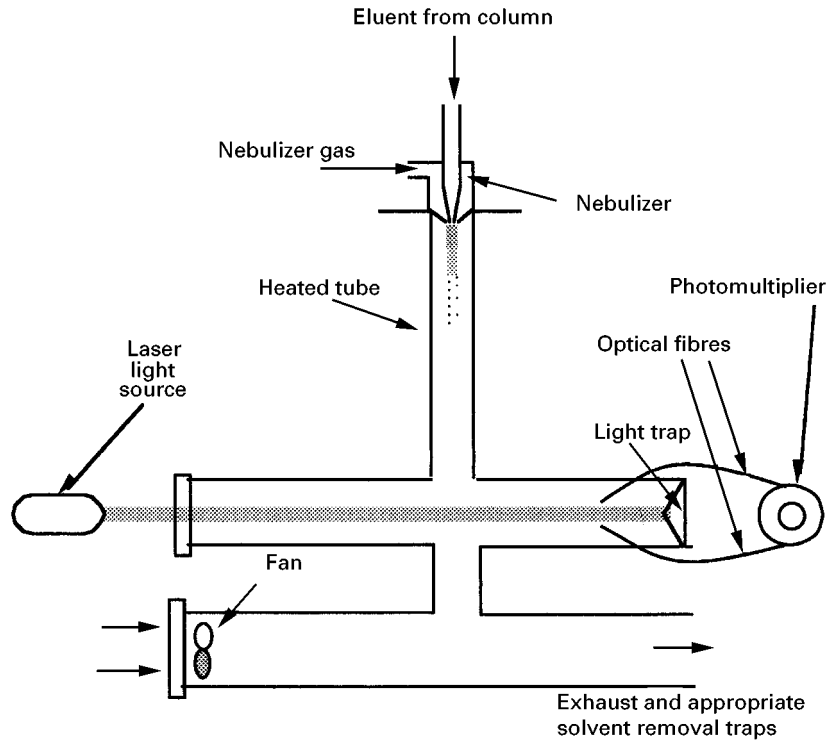

Figure 1 An evaporative light-scattering detector.

particles which in turn will be controlled by the size of the droplets generated by the nebulizer.

The average diameter of the droplets (D_0) in micron that is formed in a concentric nebulizer is given by the following equation:

$$D_0 = \frac{A\sigma_1^{1/2}}{u\rho_1^{1/2}} + B\left(\frac{\eta^1}{(\sigma_1\rho_1)^{1/2}}\right)^{0.45}\left(\frac{1000Q_1}{Q_g}\right)^{1.5} \quad [1]$$

where σ_1 is the surface tension of the mobile phase, ρ_1 is the density of the mobile phase, η_1 is the viscosity of the mobile phase, u is the relative velocity of the gas and liquid streams, Q_1 is the volume flow of mobile phase, Q_2 is the volume flow of the nebulizing gas and A and B are constants, taking values of 585 and 597.

The relationship between the mean solid particle diameter (D_s) and the mean droplet diameter (D_0) is given by:

$$D_s = D_0\left(\frac{c}{\rho_2}\right)^{1/3} \quad [2]$$

where c is the concentration of solid solute in the eluent, and ρ_2 is the density of the solid solute
Thus:

$$D_s = \left(\frac{A\sigma_1^{1/2}}{u\rho_1^{1/2}} + B\left(\frac{\eta^1}{(\sigma_1\rho_1)^{1/2}}\right)^{0.45}\left(\frac{1000Q_1}{Q_g}\right)^{1.5}\right)\left(\frac{c}{\rho_2}\right)^{1/3} \quad [3]$$

Now it is the magnitude of D_s, the number of particles per unit volume and the wavelength of the laser light that determine the intensity of light scattered at a particular angle to the incident beam.

Intensity of the Scattered Light

There are two types of scattering that can take place, Mie scattering and Raleigh scattering, both of which can take place in the present design of light-scattering detector. Raleigh scattering occurs when the particle diameter is significantly less than the wavelength of light and Mie scattering occurs when the wavelength of the light is considerably less than the particle diameter. If the nebulizer is efficient, the major effect will be Raleigh scattering and thus only this type of scattering will be considered here.

One of the early scientists to examine scattered light was Tyndall, who showed that the scattered light from small particles was polarized. He found that light scattered at right angles to the incident beam was completely linearly polarized and demonstrated that the effect was independent of the nature of the scattering media and depended only on the particles being sufficiently small.

Lord Raleigh gave a simple explanation as to why light scattered at right angles to the incident beam is completely polarized. Consider a beam of unpolarized light travelling along the x-axis impinging

on a spherical particle located at the origin of a Cartesian coordinate system. The light can be resolved into two linearly polarized components that can each act independently of the other. If the particle is small compared with the wavelength of the light, then there is a uniform instantaneous electromagnetic field over the particle. As a consequence the particle will become polarized in the direction of the field. The net result is to produce a dipole that will oscillate synchronously and in the same direction as the vibrating electric field. The oscillating dipole will radiate electromagnetic energy and this scattered radiation will be polarized in the same sense as the dipole.

Assuming the scattering direction is taken from the origin through the point defined by the polar coordinates r, θ and ϕ, then:

$$x = r\sin\theta\cos\phi; \quad y = r\sin\theta\sin\phi; \quad z = r\cos\theta \quad [4]$$

If the angle measured from the scattering direction to the dipole is ϕ then the intensity of the scattered wave at a distance r from the particle will be:

$$I = \frac{16\pi^4 a^6}{r^2\lambda^4}\left(\frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + 2\varepsilon_2}\right)\sin^2\psi \quad [5]$$

which, from the Lorenz–Lorenz law becomes:

$$I = \frac{16\pi^4 a^6}{r^2\lambda^4}\left(\frac{n^2 - 1}{n^2 + 2}\right)\sin^2\psi \quad [6]$$

Furthermore, if ϕ is 45° then:

$$I = \frac{8\pi^4 a^6}{r^2\lambda^4}\left(\frac{n^2 - 1}{n^2 + 2}\right) \quad [7]$$

It is seen that the light intensity varies inversely as the square of the distance from the particle, which would be expected from the inverse-square law. Not so obvious is the inverse dependence of the scattered light intensity on the fourth power of the wavelength of the incident light. In practice, the equation does not precisely predict the intensity of the scattered light as there are a significant number of particles present that are not greatly smaller than the wavelength of the incident light.

The Performance of the Light-scattering Detector

The evaporative light-scattering detector has two major advantages over many other liquid chromatography detectors. Firstly, like all transport detectors, its function is almost completely independent of the solvent used for chromatographic development, with the one proviso that all the solvents used must be sufficiently volatile. This provides a wide range of solvent choice, allowing unique solvents to be used that would be impossible with other types of detectors. Its second advantage is its catholic response, which is similar to that of the refractive index detector. Moreover, as opposed to the refractive index detector, the evaporative light-scattering detector readily tolerates gradient elution development.

However, there are also certain disadvantages to this type of detector and certain precautions that need to be taken in its operation. One safeguard is to use a 0.45 μm filter in line with the nebulizing gas supply to remove any dust particles that may get caught up in the gas flow. Foreign particles in the nebulizing flow will contribute noise to the system and, as a consequence, reduce the sensitivity or increase the minimum detectable concentration. In addition, the nebulizer and drift tube will need to be cleaned regularly to remove accumulated sample deposits. This should be carried out every few weeks: failing to do this will not only result in significantly increased noise, but also adversely affect analytical reproducibility.

Occasionally the central jet of the nebulizer carrying the column eluent will become blocked, particularly if high solute concentrations or sticky solutions are nebulized. A blocked nebulizer tube will result in increased back-pressure and, if another detector is employed prior to the evaporative light-scattering detector, then the increased pressure can burst the sensor cell. A pressure sensor should be placed prior to the nebulizer so the back-pressure can be continuously monitored. If this pressure suddenly increases above the normal operating pressure, then the nebulizer will need to be disassembled and cleaned. A relief valve fitted behind the nebulizer will also protect any other detector that is being used from damage.

The nonlinear response of the evaporative light-scattering detector is a more serious problem as it renders quantitative analysis more involved. Furthermore, as the response varies between different solutes, calibration curves must be produced for each substance that is to be determined. The results are usually curve-fitted to an appropriate polynomial or power function which can then be used to modify the peak height or peak area measurements obtained in the actual analysis.

In general, the response of the detector can be fitted to the equation:

$$y = ac^b \quad [8]$$

DropBooks

where y is the detector response, c is the concentration of solute in the eluent, a and b are constants.

Consequently, the curve relating log y against log c will be linear and the slope will provide the value of b. In practice, b is usually found to be less than 2, which is the value it would be if only Raleigh scattering was taking place.

The two main disadvantages to the evaporative light-scattering detector are its relatively poor sensitivity (or high minimum detectable concentration) and its nonlinear response to the concentration of solute. There are a number of different commercial detectors of this type available and the consensus of opinion is that the sensitivity (or minimum detectable concentration) is similar to that of the refractive index detector, i.e. about 3×10^{-6} g mL^{-1}. This sensitivity compares unfavourably with that of the fixed-wavelength UV detector, *c.* 5×10^{-8} g mL^{-1}, the fluorescence detector, *c.* 1×10^{-9} g mL^{-1} and that of the modified moving ribbon transport detector, *c.* 8×10^{-8} g mL^{-1}. Nevertheless, the sensitivity of 3×10^{-6} g mL^{-1} is quite practical for use in liquid chromatography and, due to its near universal response and its solvent independency, the detector is popular for lipid analysis and for other materials that do not fluoresence or have UV chromatophores.

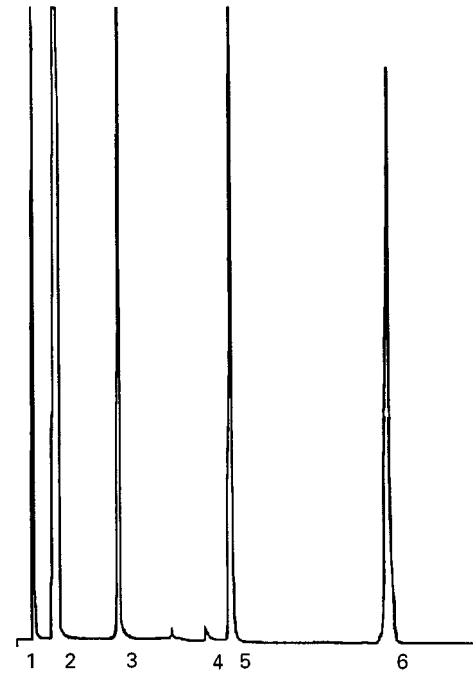

Figure 2 The separation of some lipid-class materials monitored by an evaporative light-scattering detector. For key, see Table 1.

Applications of the Light-scattering Detector

Some examples of the use of the light-scattering detector to monitor the separation of materials that normally require gradient elution for resolution, but are sometimes difficult to sense by other types of detector, are lipids, fatty acids and phospholipids. An example of a chromatogram obtained from a sample containing a mixture of general lipid-class solutes and monitored by the light-scattering detector is shown in **Figure 2** (**Table 1**).

The sample size is rather high for general quantitative liquid chromatographic analyses but the column does not appear to be overloaded. The minimum detectable mass estimated from this chromatogram appears to be about 10 ng of solute. To some extent, this detector provides an alternative to the conventional transport detector as it detects all substances irrespective of their optical or electrical properties. However, modern versions of the conventional wire or ribbon transport detector are reported to have significantly greater sensitivity.

Figure 3 depicts the separation of a mixture of fatty acids. The C_{18}-bonded silica column was 25 cm long, 2.1 mm i.d. and packed with particles of 3 μm diameter. The flow rate was 0.4 mL min^{-1} and the solvents used were water and acetonitrile. The gradient employed is shown in **Table 2** and is typical for a reversed-phase column.

The solutes are initially retained by dispersive forces between the solutes and the stationary phase and are progressively eluted as the dispersive character of the mobile phase is increased with the greater concentration of acetonitrile. The weights quoted appear to be the concentration of each solute in the sample injected 20 μL of solvent. It is seen that an excellent response is obtained and the chromatogram is quite suitable for accurate quantitative analysis.

The separation of some phospholipids is shown in **Figure 4**. The column was 10 cm long, 4.6 mm i.d. and packed with particles of silica 3 μm in diameter.

Table 1 Key to Figure 2

Peak	*Compound*	*Mass (μg)*	*Retention time (min)*
1	Cholesterol ester	5	0.717
2	Triglyceride	18	1.746
3	Cholesterol	10	4.687
4	Unknown		8.860
5	Phosphatidyl choline	10	10.028
6	Phosphatidylethanolamine	10	17.390

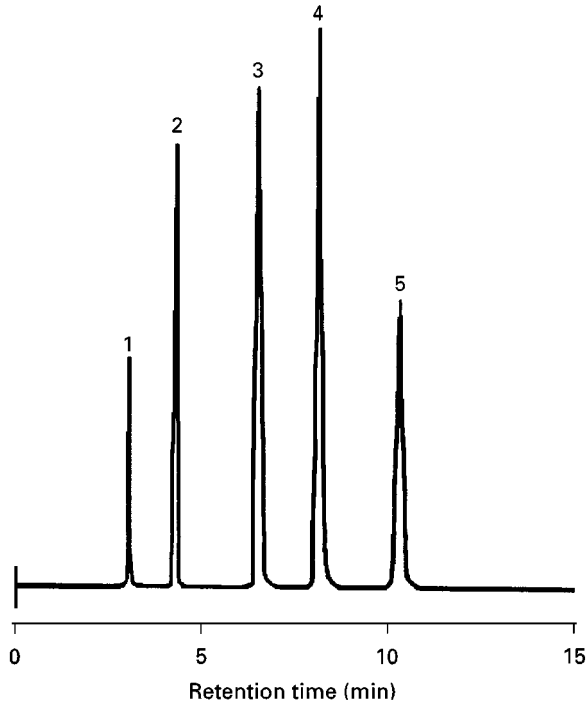

Figure 3 The separation of some fatty acids monitored by an evaporative light-scattering detector. Peaks: 1, capric acid (0.10 mg mL^{-1}); 2, lauric acid (0.03 mg mL^{-1}); 3, myristic acid (0.03 mg mL^{-1}); 4, pentadecanoic acid (0.02 mg mL^{-1}); 5, palmitic acid (0.03 mg mL^{-1}).

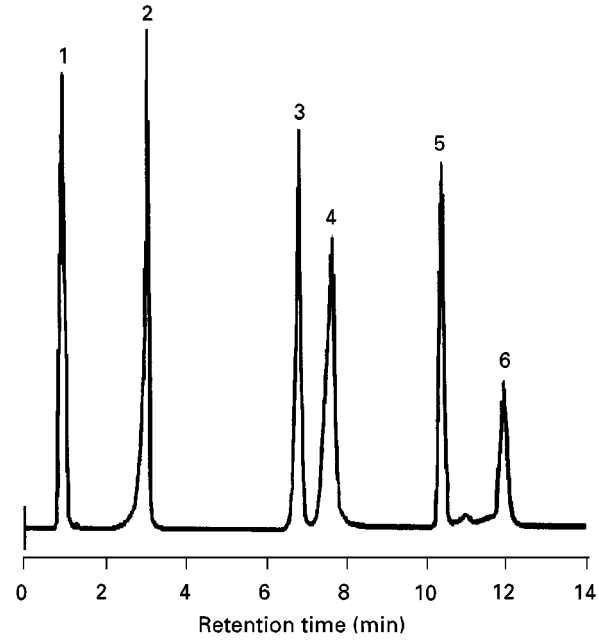

Figure 4 The separation of some phospholipids monitored by an evaporative light-scattering detector. Peaks: 1, cholesterol (0.15 mg mL^{-1}); 2, palmitic acid (0.25 mg mL^{-1}); 3, phosphatidylethanolamine (0.15 mg mL^{-1}); 4, phosphatidylserine (0.30 mg mL^{-1}); 5, phosphatidylcholine (0.15 mg mL^{-1}); 6, sphingomyelin (0.15 mg).

DropBooks

The flow rate was 1.25 mL min^{-1} and the solvents used were water, isopropanol and *n*-hexane. The gradient employed is shown in **Table 3** and has obviously been specially developed for this type of separation on silica gel.

In this separation the solutes are largely retained by polar forces and are progressively eluted by increasing the proportion of isopropanol and water. The strong polar solvents deactivate the stationary phase by preferential adsorption and this allows the strong dispersive forces between the solutes and the hexane to elute the solutes. Again, the weights quoted appear to be the concentration of each solute in the sample injected in 20 μL of solvent. It is clear that the detector is quite sensitive to these solutes and, again, the response and resolution are more than adequate for accurate quantitative analysis.

Table 2 Gradient for a typical reversed-phase column with solvents A (water) and B (acetonitrile)

	0 min	*5 min*	*10 min*	*20 min*
% B	77	80	90	95

Table 3 Gradient using solvents A (isopropanol), B (*n*-hexane) and C (water)

	0 min	*7 min*	*15 min*
% A	58	52	52
% B	40	40	40
% C	2	8	8

Conclusions

Although the evaporative light-scattering detector is mechanically somewhat clumsy, relatively expensive and has a nonlinear response and limited sensitivity, it still fills a need for an effective detector that can be used for certain classes of compounds that cannot be sensed by other detector types. In addition, as opposed to the refractive index detector that can also sense similar materials, it allows a free choice of solvent and easily tolerates solvent programming.

See also: **II/Chromatography:** Detectors: Laser Light Scattering. **III/Lipids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography.

Further Reading

Charlesworth JM (1978) Evaporative analyzer as a mass detector for liquid chromatography. *Analytical Chemistry* 50: 1414.

Kerker M (1969) *The Scattering of Light and other Electromagnetic Radiation*. New York: Academic Press.

MacRae R and Dick J (1981) Analysis of carbohydrates using the mass detector. *Journal of Chromatography* 210: 138–145.

Mourey TH and Oppenheimer LE (1984) Principles of operation of an evaporative light-scattering detector for liquid chromatography. *Analytical Chemistry* 56: 2427–2434.

Oppenheimer LE and Mourey TH (1985) Examination of the concentration response of evaporative light-scattering mass detectors. *Journal of Chromatography* 323: 297–304.

Stolyhwo A, Martin M and Guiochon G (1987) Analysis of liquid classes by HPLC with the evaporative light scattering detector. *Journal of Liquid Chromatography* 1243–1253.

Detectors: Fluorescence Detection

R. P. W. Scott, Avon, CT, USA

Copyright © 2000 Academic Press

The process whereby molecules are excited by electromagnetic radiation to produce luminescence is termed photoluminescence. If the release of energy is delayed, or persists after the removal of the exciting radiation, then the substance is said to be *phosphorescent*. Signal persistence (even with a short but significant lifetime) limits the use of phosphorescence for liquid chromatography (LC) detection, because signal continuance will produce apparent peak broadening and consequent loss of resolution. If the release of electromagnetic energy is immediate, or stops on the removal of the excitation radiation, the substance is said to be *fluorescent*. In contrast to phosphorescence, fluorescence has been shown to be extremely useful for LC detection, and has provided some of the highest sensitivities available.

When light is absorbed by a molecule, a transition to a higher electronic state takes place and this process is highly specific for each substance. This is because radiation of a particular wavelength, or energy, will be absorbed by specific molecular structures. If electrons are raised, due to absorption of light energy, to an upper excited singlet state, and the excess energy is not dissipated rapidly by collision with other molecules or by other means, the electron will return to the ground state with the emission of light at a lower frequency. Under such circumstances the substance is said to fluoresce. In reality, some energy is always lost before emission occurs and thus, in contrast to Raman scattering, the wavelength of the fluorescent light emitted is always greater than the incident light. For further information on the theory of fluorescence the reviews by Guilbault, Undenfriend and Rhys-Williams are recommended (see Further Reading section).

With the exception of certain electrochemical detectors and the mass spectrometer, the fluorescence detector affords greater sensitivity to sample concentration than other devices. In addition, the fluorescence sensor is less sensitive to changes in ambient conditions, e.g. temperature and pressure. The high sensitivity that is achieved is also partly due to the very low background light level and the consequent low noise level. The low noise level of the fluorescent detector is in contrast to those detectors that involve light absorption measurements, where the signal is superimposed on a strong background signal with a high noise level. The major disadvantage of fluorescence detection is that relatively few compounds fluoresce in a practical range of wavelengths. However, the scope of fluorescence detection can be extended by forming derivatives. For example, the reagents fluoropa (*o*-phthalaldehyde) and fluorescamine (4-phenyl-spiro(furan-2-(3H),1′-phthalan)-3′,3′-dione) are both commercially available derivatizing reagents that can react with primary amines to produce fluorescent derivatives. One other minor disadvantage is the effect of molecular oxygen which, if present in the mobile phase, can cause significant fluorescent quenching. It is essential, therefore, for maximum and constant response, to degas the solvents by helium sparging before use.

Most fluorescent detectors are configured so that the fluorescent light that is sensed is emitted at an angle (usually at right angles) to the direction of the exciting incident light beam. This arrangement minimizes the amount of incident light that may provide a background signal to the fluorescent sensor. It follows that the fluorescent signal is sensed against